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Campylobacter concisus pseudo-outbreak caused by improved culture conditions

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Abstract

An unusual increase of *Campylobacter concisus* in stool cultures provoked an outbreak investigation at the University Hospital of Bern. No epidemiological links were found between cases, and the *Campylobacter* isolates were clonally unrelated. A change in culture conditions to a hydrogen-rich atmosphere enhancing growth of *C. concisus* was deemed responsible for this pseudo-outbreak.

Text

Campylobacter concisus is a fastidious *Campylobacter* species whose pathogenic role in human disease is not established. Isolation of *C. concisus* in respective samples has been reported in periodontal disease, Barrett's esophagus (1, 2), enteritis, and inflammatory bowel disease (IBD) (3), and the pathogen has been proposed to be linked to certain hepatobiliary and kidney conditions in children (4). High prevalences of *C. concisus* in stool samples were not only encountered in children and adults suffering from diarrhea (detection rate: 0.7- 49%) but also in healthy controls (detection rate: 0-52%) (1, 3). Immunodeficiency (5) and age extremes (6, 7) appear to be determinants of higher prevalence in stool. Moreover, *C. concisus* could be detected by PCR almost universally in human saliva samples (3). Thus it is unresolved whether *C. concisus* is merely a commensal of the human digestive tract or a true pathogen. In light of its genetic variability both may be true (1, 2). In late 2013, a substantial increase in the number of stool cultures positive for *C. concisus* was observed at the Bern University Hospital. In order to rule out an outbreak, an epidemiological investigation was conducted.

Bern University Hospital is a 950-bed tertiary care teaching hospital in Switzerland. In the microbiology laboratory, approximately 2,000 stool samples are cultured for enteropathogenic bacteria each year. For *Campylobacter* cultures, clinical stool specimens were inoculated onto Preston agar plates and incubated in a microaerobic atmosphere at 35°C and 42°C, respectively, for 48 hours. Microaerobic conditions were obtained with gas generator packs (CampyGen, Oxoid, UK) producing a final atmosphere of 5% O_2, 10% CO_2 and 85% N_2, or with evacuation and gas replacement of anaerobic jars (TRILAB, Jenny Science, Switzerland) containing approximately 5% O_2, 8% CO_2, 15% H_2 and 72% N_2 (replacing 76% of the air with an anaerobic gas mixture containing 70% N_2, 20% H_2 and 10% CO_2). Isolates were identified by matrix-assisted laser

desorption/ionization time-of-flight mass spectrometry (Bruker Biotyper MALDI-TOF/MS, Bruker Daltonics, Bremen, Germany) and sequence analysis using the MicroSeq®500 16S rDNA PCR and Sequencing Kits (Applied Biosystems, Foster City, CA). Genetic relatedness of isolates was analyzed by repetitive extragenic palindromic PCR (rep-PCR) (8). Cases were defined as all patients with *C. concisus* isolated from stool samples between 2003 and 2013. Retrospective and prospective case finding was performed including patients meeting the case definition during 2013. Incidence data were taken from electronic data on all samples processed at the microbiology laboratory. The laboratory incidence was defined as number of *C. concisus* identifications divided by the total number of stool cultures processed in the given time period. Epidemiological and clinical data were taken from the hospital's electronic patient chart (CGM Phoenix, Parametrix Solution, Lachen, Switzerland), primarily focusing on acquisition mode (nosocomial vs. community-acquired). Nosocomial acquisition was defined as diagnosis >48 hours after hospital admission. Patients diagnosed as outpatients with hospitalization within the previous month were considered to have nosocomial *C. concisus* (3, 9). This outbreak investigation was part of the infection prevention mandate and therefore not subject to review by the ethics committee.

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In the decade prior to the increase *C. concisus* was rarely detected in routine stool cultures (on average 1.1 isolates annually). In 2013 *C. concisus* was isolated from stool specimens of 21 individual patients and from an intestinal biopsy of another patient. In all instances, *C. concisus* was the sole organism with pathogenic potential detected. The incidence increased from an average of 0.03 % (1/2012- 5/2013) to 1.92% (June-December 2013); $p<0.001$, chi-square test (Fig. 1).

Mean age of the 22 patients included in the analysis was 46.7 years (SD±25.9 years, range: 3 months-85 years). Eleven of 22 patients were female. Eight of 22 patients were outpatients. In 8/14 inpatients *C. concisus* was detected >48 hours after the first admission and in 3/14 patients more than 48 hours into the admission, during which the diagnosis was made. Two patients (#3 and #5) were hospitalized on the same ward during the same time period prior to *C. concisus* detection, with patient #3 being on contact precautions due to diarrhea of unknown etiology. Prior to detection of *C. concisus*, 3/22 patients had colonoscopy at our hospital and 1/22 at an external hospital (with intervals of 1, 4, 122, and 140 days prior to diagnosis). Two patients had colonoscopy on the same ward but months apart. In one additional patient, *C. concisus* was cultured from biopsy material. Putative risk factors for colonization/infection were found in 13/22 patients [immunodeficiency=6 (3 with IBD); extremes of age=6; extremes of age and immunodeficiency=1]. Seven of 22 cases suffered from either IBD (n=4) or chronic kidney disease (n=3), among which 4/7 cases were also immunodeficient. Fig. 2 summarizes epidemiological data and the results of rep-PCR-based genotyping.

After reviewing the cases, a change in microaerobic culture conditions was identified as the most likely explanation for the putative outbreak. Shortly before the *C. concisus* incidence started to increase, an automated system for the evacuation and gas replacement of anaerobic jars had been introduced. In contrast to the previously used microaerobic gas generator packs, which do not produce hydrogen, the resulting atmosphere of the new system contained approximately 15% hydrogen. Some *Campylobacter* species, such as *C. concisus*, appear to require increased hydrogen concentrations for optimal growth (10). When subculturing five frozen *C. concisus* isolates (not the original stool samples) from the study period under both culture conditions, only weak or no growth was encountered with the previous methodology (Fig. 3).

In conclusion, a pseudo-outbreak of *C. concisus* due to a change in laboratory procedures was identified. A pseudo-outbreak is defined as an episode of increased disease incidence

due to enhanced surveillance or other factors but not related to the disease under study (11). Except for one patient, no epidemiological links suggesting nosocomial transmission were found. In addition, genotyping revealed no close relationship between the isolates available for testing. Unfortunately, the isolate of the first – and potential index - case (#3) was not available for genotyping**.** The introduction of a new microaerobic culture system containing a high hydrogen concentration compared to conventional microaerobic conditions presumably led to a better recovery of *C. concisus* from fecal samples. The clinical significance of *C. concisus* remains unclear to date but may be easier to determine as diagnostic procedures improve and permit the differentiation between pathogenic and non-pathogenic strains.

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Figure legends

Figure 1: (A) Annual number of clinical samples and patients positive for *C. concisus* from 2003 to 2013. (B) Absolute numbers (squares) and incidence (solid line) of *C. concisus* isolates from January 2012 to December 2013. The arrow indicates the introduction of the new culture method.

Figure 2: Results of genotyping and epidemiologic data of all 22 patients diagnosed with *C. concisus* in stool samples taken in 2013. One strain was isolated from an intestinal biopsy (patient #17). Patients are numbered in the order of collected culture. A strain (X) isolated in 2010 was included as unrelated control for typing purposes. NA, not available; m, male; f, female

Figure 3: *C. concisus* isolate subcultured under previous (A, gas generator pack, only few pinpoint colonies visible (arrow)) and new culture conditions (B, anaerobic jar supplemented with hydrogen) for 3 days at 42°C.

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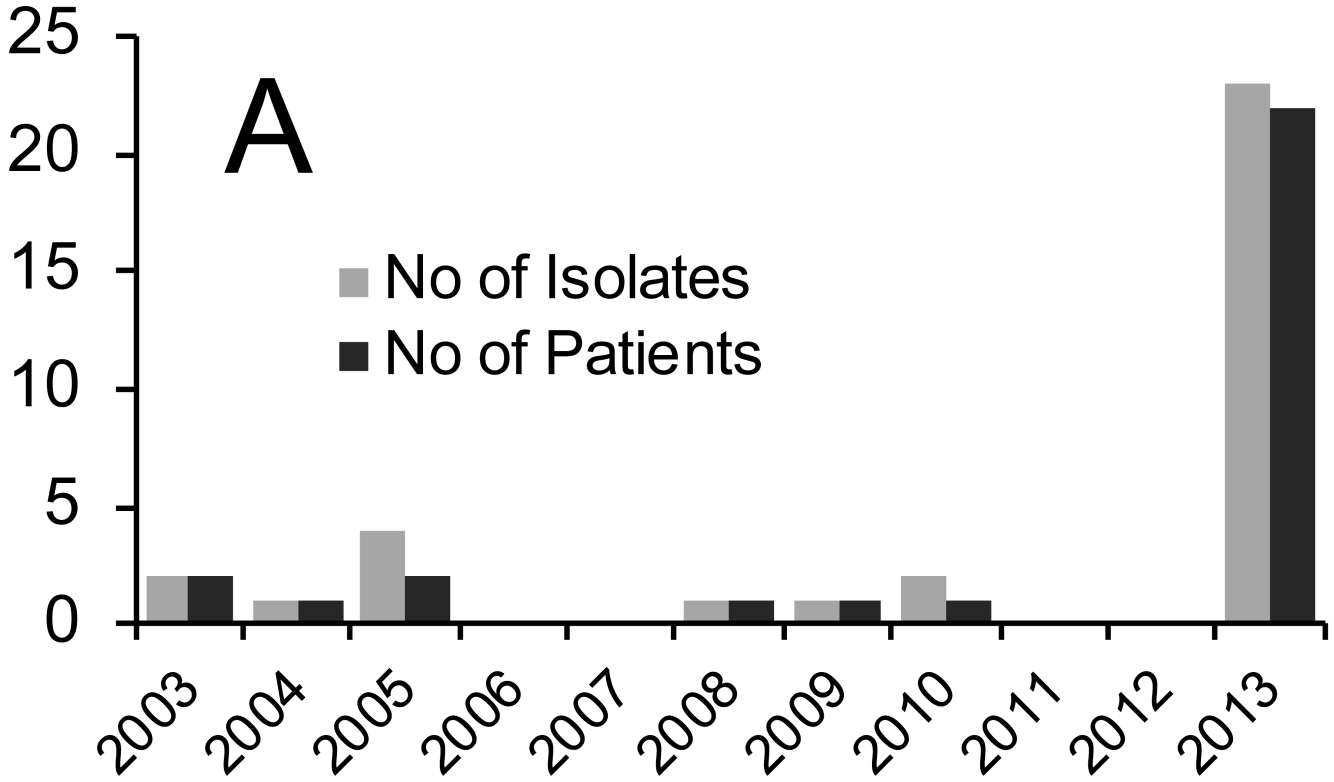

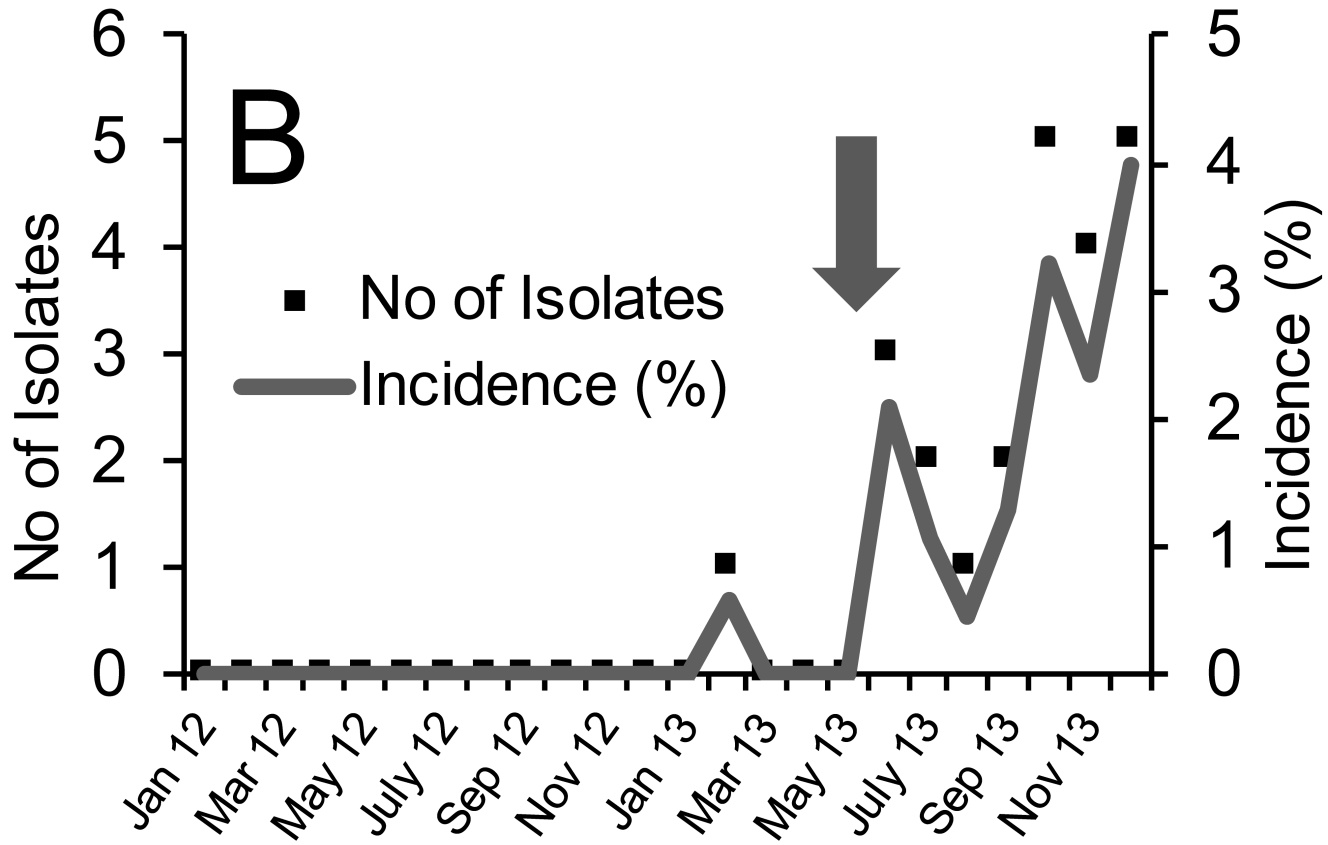

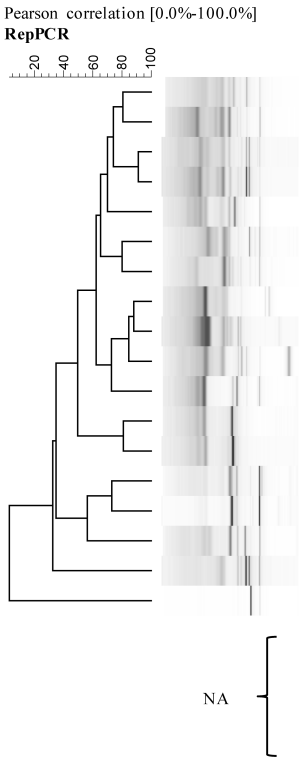

Patient	Age/Sex	Ward	Symptoms onset >48h after admission (n of days)	Overlapping hospitalisation with other patients (patient number)	Date of positive stool sample	Colonoscopy prior to positive culture (n of days)
19	68/f	Surgery 2/ IMC 1/ Surgery 3	No	Yes (14, 18, 20, 21, 22)	16.12.	No
20	64/m	Medical 4	No	Yes (19, 21)	18.12.	No
11	73/m	Medical 3/ Medical 4	No	Yes (9, 10, 12, 13)	2.10.	External (140)
17	21/f	Outpatient 3	No	Outpatient	19.11.	Yes (0)
12	33/f	Emergency	No	Outpatient	3.10.	No
8	76/m	Outpatient 2/ Medical 1	No	Outpatient	23.9.	No
9	65/m	Medical 2	Yes (7d)	Yes (5, 8, 10, 11, 12, 13, 17)	23.9.	No
16	79/f	Medical 6/ Outpatient 3	No	Yes (10, 14)	12.11.	No
21	3mths/f	Pediatric 1	No	Yes (19, 20, 22)	23.12.	No
15	27/f	Emergency	No	Outpatient	9.11.	No
5	9/m	Pediatric 1/ Pediatric 2	No	Yes (3, 4, 6, 7, 8, 9, 10)	2.7.	No
7	62/f	Surgery 1	Yes (4d)	Yes (5, 10)	24.8.	No
14	24/m	Medical 5	Yes (7d)	Yes (10, 16, 18, 19)	22.10.	No
X	28/m	NA	NA	No	2010	NA
18	49/m	Medical 7	Yes (6d)	Yes (14, 19)	29.11.	No
10	17/m	Pediatric 3	No	Yes (5, 7, 9, 11 - 17)	2.10.	Yes (1)
22	85/m	Medical 3	No	Yes (19, 21)	23.12.	No
13	36/f	Outpatient 3	No	Outpatient	3.10.	No
1	64/f	Outpatient 1	No	Outpatient	19.2.	No
2	46/f	Outpatient 2	No	Outpatient	4.6.	Yes (5)
3	7/f	Pediatric 1	No	Yes (4, 5)	18.6.	No
4	69/m	ENT ward/ Outpatient 1	No	Yes (3, 5)	18.6.	No
6	54/m	Outpatient 1	No	Outpatient	16.7.	Yes (122)